DNA Sequence Analysis and Restriction Fragment Length Polymorphism (RFLP) Typing of the HLA-DQw2 Alleles Associated with Dermatitis Herpetiformis

Clark C. Otley, Richard J. Wenstrup, and Russell P. Hall

Division of Dermatology, Department of Medicine and Division of Cardiology (RJW), Department of Pediatrics, Duke University Medical Center and Durham VA Medical Center, Durham, North Carolina, U.S.A.

Dermatitis herpetiformis (DH) is a blistering autoimmune skin disease associated with a 95–100% incidence of the HLA class II antigen HLA-DQw2. Although the precise role of this antigen in the pathogenesis of DH is unclear, one theory proposes that patients with DH possess a molecularly unique subtype of the HLA-DQw2 antigen that causes immune abnormalities eventuating in the clinical manifestations of DH. To test this hypothesis, we performed DNA sequence analysis on the highly polymorphic HLA-DQB1 and HLA-DQA1 loci of eight patients with dermatitis herpetiformis. All DQB1 alleles sequenced were identical to the previously described HLA-DQB*0201 allele from HLA-DQw2 normal subjects. In addition, DQA1 alleles sequenced were identical to those alleles previously associated with HLA-DQw2 (DQA*0201, DQA*0501). These data document that although HLA-DQw2 appears to be a necessary element in the pathogenesis of DH, the development of DH is not dependent on the presence of a unique HLA-DQw2 antigen. HLA-DQ allelic typing by restriction fragment length polymorphism analysis of PCR-amplified HLA-DQA1 and HLA-DQB1 fragments was also performed in ten patients with DH to determine the allelic distribution among both HLA-DR3 (eight patients) and non-DR3 (two patients) DH patients. At the HLA-DQ β chain locus, all patients possessed the DQB1*0201 allele. At the HLA-DQ α chain locus, all HLA-DR3 patients and one non-DR3 patient displayed a pattern consistent with the DQA1*0501 allele, whereas one non-DR3 patient displayed a pattern consistent with the DQA1*0201 allele. These data document that patients with DH do not express a unique HLA-DQw2 heterodimer, that the HLA-DQw2 molecules present in patients with DH have no DNA sequence differences from those found in normal HLA-DQw2 subjects and therefore that susceptibility to DH is not due to a unique HLA-DQw2 molecule. *J Invest Dermatol 97:318–322, 1991*

Dermatitis herpetiformis (DH) is a pruritic, papulovesicular skin disease characterized by granular IgA deposits at the dermal-epidermal junction, an associated gluten-sensitive enteropathy (GSE), and a strong association with specific human leukocyte antigens (HLA) [1]. Although HLA associations have been demonstrated in a variety of diseases, including insulin-dependent diabetes mellitus (IDDM), ankylosing spondylitis, gluten-sensitive enteropathy (GSE), and psoriasis, the exact role of genetic factors in disease pathogenesis remains uncertain [2–9]. Both dermatitis herpetiformis and isolated gluten-sensitive enteropathy have exceptionally strong associations with the HLA class II region, located within the major histocompatibility complex on the short arm of chromosome six [10–13].

Patients with dermatitis herpetiformis have an increased frequency of the HLA antigens HLA-A1, -B8, -DR3, -DQw2, and -DPw1. Serologic typing of patients with DH has revealed that 75–90% express the HLA class I antigens HLA-A1 and -B8, whereas 95–100% express the HLA class II antigens HLA-DR3 and -DQw2 [11–18]. Further analysis of these HLA associations showed that the HLA class I and HLA-DP associations found in patients with DH are due to linkage disequilibrium of these antigens with the HLA-DR3 and -DQw2 antigens [12,19,20] These data document that the strongest association found in patients with DH is with the HLA class II antigen HLA-DQw2, present in 100% of a recent series of 43 patients with DH [20]. Virtually identical HLA profiles have been observed in patients with isolated gluten-sensitive enteropathy [21,22].

The HLA class II region is composed of three known subregions that encode for the cell-surface molecules HLA-DP, HLA-DQ, and HLA-DR. HLA class II molecules are heterodimeric glycoproteins, formed by an α and a β chain ($\alpha_1\beta_1$) [23,24]. HLA-DQw2 is the designation for the serologically defined HLA class II molecule studied. Each class II subregion contains multiple loci representing both expressed and non-expressed (pseudo genes) α and β chain genes. These genes of the D region are all preceded by the letter D, followed by a letter designating the subregion (HLA-DQ, -DR,

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Reprint requests to: Dr. Russell P. Hall, Box 3135, Duke University Medical Center, Durham, NC 27710.

Abbreviations:
DH: dermatitis herpetiformis
GSE: gluten sensitive enteropathy
HLA: human leukocyte antigen
IDDM: insulin dependent diabetes mellitus
RFLP: restriction fragment length polymorphism

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-DP, etc.), followed by an A or B, for α or β chain, and finally by a number, designating the specific gene [25]. Thus, the DQ α chain gene that is expressed is designated DQA1 whereas the expressed DQ β chain gene is designated DQB1. Finally, specific alleles of the HLA-DQA1 and HLA-DQB1 genes are designated DQA1*0101, DQA1*0201, DQB1*0401, DQB1*0201, etc. With the exception of the DR and DP α chains, HLA class II molecules are highly polymorphic, with multiple allelic variants possible at each locus. The particular combination of HLA class II α and β chain genes may determine, in part, the immune responsiveness of an individual to foreign antigens and also may influence susceptibility to autoimmune disease [26–29].

Despite the strong HLA class II associations found in patients with DH it is clear that additional factors must contribute to the pathogenesis of DH, because 40% of normal subjects also express HLA-DQw2 [20]. One explanation of this observation is that patients with DH may express unique HLA class II antigens which, although identical by serologic techniques to normal class II antigens, are encoded for by a unique DNA sequence of the HLA α or β chain genes. Alterations in the polymorphic second exon of the HLA α or β chain genes would be of particular interest because this region encodes the putative antigen-binding sites of the HLA molecules [8]. Subtypes of HLA molecules have previously been found in patients with several autoimmune diseases, including pemphigus vulgaris and IDDM [30–33].

In order to determine if patients with dermatitis herpetiformis have a unique HLA-DQw2 molecule distinct from that found in normal HLA-DQw2 subjects, DNA sequence analysis was performed on the highly polymorphic second exons of the HLA-DQ α (DQA1) and β chain (DQB1) genes of patients with DH [33–36]. Sequences were then compared to previously described DQB1 and DQA1 gene sequences from HLA-DQw2 normal subjects as well as sequences from HLA-DQw2 patients with isolated gluten-sensitive enteropathy [37–39]. In order to confirm and extend our sequencing data, HLA-DQ typing by restriction fragment length polymorphism (RFLP) analysis was performed [40–42].

MATERIALS AND METHODS

Patients Ten patients with dermatitis herpetiformis were studied. The diagnosis of dermatitis herpetiformis was made by the presence of typical clinical features of DH and direct immunofluorescence examination of perilesional skin showing granular deposits of IgA at the dermal-epidermal junction. Patients were HLA typed by standard microcytotoxicity techniques and all expressed HLA-DQw2, whereas eight of ten expressed HLA-DR3 as previously reported [20,43]. Patients PT and PB are American blacks; the remainder are caucasian.

Polymerase Chain Reaction (PCR) Genomic DNA was isolated from Epstein-Barr virus-transformed lymphoblastoid B-cell lines from ten dermatitis herpetiformis patients by methods previously described [44]. One microgram of genomic DNA was enzymatically amplified in a 100-μl polymerase chain reaction using DQB1 and DQA1-specific oligonucleotide primers (GH28/29,GH26/27) [33–36,45]. These primers are located at the 5′ and 3′ ends of the polymorphic second exon of the HLA-DQ α and β chain genes and have been previously described [33,45]. The conditions for the PCR reaction were denaturation at 94°C for 1 min and 10 seconds, annealing at 50°C for 2 min and 10 seconds, and extension at 72°C for 3 min and 10 seconds. Twenty-five cycles of amplification were performed followed by a final extension reaction for 7 min at 72°C.

Cloning Amplified HLA-DQB1 and -DQA1 fragments wre digested with BamHI and PstI, isolated by gel electrophoresis in 4% NuSieve agarose (FMC BioProducts), and ligated into pUC18 plasmid vectors using T4 DNA Ligase (New England BioLabs). DH5α *Escherichia coli* (Bethesda Research Laboratories) were transformed with the recombinant plasmids and clonal plasmids were extracted by alkaline lysis. On average, nine clones from two separate PCR reactions were isolated per patient.

DNA Sequencing The recombinant plasmids were sequenced by the dideoxy chain-termination method of Sanger using ^{35}S-dATP and Sequenase 2.0 DNA polymerase (US Biochemical) [46]. DNA sequences were compared to available HLA-DQB1 and -DQA1 sequences [25,37–39].

HLA-DQ Typing by RFLP Analysis Genomic DNA was amplified by the polymerase chain reaction using DQB1 and DQA1 specific primers as described above. Amplified DQB1 and DQA1 fragments were isolated by gel electrophoresis in 4% NuSieve agarose as described above. The band containing the amplified DNA was excised and the DNA was purified using Geneclean (Bio 101). The DQA1 and DQB1 fragments were digested with the restriction endonucleases HaeIII, DdeI, and RsaI (DQA1), and HhaI and HaeIII (DQB1). Digestion products were subjected to non-denaturing 15% polyacrylamide gel electrophoresis in TBE buffer at 140V for 18 h. The RFLP patterns observed were compared to those obtained from HLA-DQB1 and HLA-DQA1 sequences previously reported and allelic assignments were made [40–42]. For example, digestion of the HLA-DQA1*0201 fragment with RsaI, results in 222 base pair and 20 base pair fragments whereas the HLA-DQA1*0501 allele is digested into three pieces (195 bp, 27 bp, 20 bp). By comparison of the patterns derived from different enzyme digestions the identity of specific alleles can be deduced [40–42].

RESULTS

HLA-DQB1 and HLA-DQA1 Sequence Analysis The nucleotide sequences of both the HLA-DQ β chain (DQB1) second exon and -DQ α chain (DQA1) second exon in patients with dermatitis herpetiformis were compared to known sequences from normal, unaffected HLA-DQw2 controls [25,37–39]. Previously published normal DQw2-associated sequences include a single allele at the DQB1 locus, DQB1*0201, and two allelic variants at the DQA1 locus, DQA1*0501 and DQA1*0201 [25,37–39]. DNA sequence analysis of the DQB1 locus associated with the HLA-DQw2 antigen in eight of eight patients with DH was identical to the previously published allele of normal HLA-DQw2 subjects, DQB1*0201 (Table I) [25,37–39]

Two DQA1 sequences have been previously reported to be associated with the DQw2 antigen in normal subjects. DQA1*0201 has been found predominately in subjects who are HLA-DQw2 in association with HLA-DR7, whereas DQA1*0501 has been found predominately in HLA-DQw2 normal subjects who also express HLA-DR3. DNA sequence analysis of the DQA1 locus associated with HLA-DQw2 was performed in two patients with DH. The DNA sequences obtained were identical to those found in normal HLA-DQw2 subjects, namely, DQA1*0201 and DQA1*0501 (Table I).

Characterization of DQA1 and DQB1 Loci by RFLP Analysis After sequence analysis of the DQw2-associated DQB1 locus in eight patients and the DQw2-associated DQA1 locus in two patients revealed no abnormality of DNA sequence we elected to complete the characterization of the patients with DH by RFLP analysis of PCR-amplified DQA1 and DQB1 fragments [40–42]. This technique enabled us to determine if patients were homozygous for the DQB1*0201 allele and to determine the associated DQA1 allele in the remaining patients without DNA sequence analysis.

HLA-DQB1 Locus As expected, RFLP analysis of the DQB1 locus in eight of eight patients with DH displayed an RFLP pattern consistent with the DQB1*0201 allele [40–42]. The RFLP pattern obtained also demonstrated the presence of a second allele, different than DQB1*0201, in all eight patients. Thus all patients were heterozygous at the DQB1 locus, expressing DQB1*0201 plus another undefined DQB1 allele (Table I). It is important to note that both patients who were determined to express only HLA-DQw2 by serologic techniques also were found to be heterozygotes for DQB1, demonstrating the presence of a serologically uncharacterized

Table I. DNA Sequence and RFLP Analysis of the HLA-DQw2-Associated DQA1 and DQB1 Loci in Patients with DH[a]

Patient	DQ Serotype	DR Serotype	DQB1 RFLP Type	DQA1 RFLP Type	DQB1 Sequence	DQA1 Sequence
PSA	w2,	3,7	0201	0501[b], 0201[c]	0201	0201
PDE	w2,w1	3,w6	0201	0501[b]	0201	ND[e]
PC6	w2,w1	3,w6	0201	0501[b]	0201	ND
PA	w2,w3	3,4	0201	0501[b]	0201	0501
PDU	w2,w1	3,w6	0201	0501[b]	0201	ND
PG1	w2,w1	3,w6	0201	0501[b]	0201	ND
PB	w2,w1	3,1	0201	0501[b]	0201	ND
PT	w2,	3,7	0201	0501[b], 0201[c]	0201	ND
PS1	w2,w1	4,w6	ND	0201[d]	ND	ND
PT2	w2,w3	5,7	ND	0501[b]	ND	ND

[a] Serotypes were previously obtained and are published in [26].
[b] DQA1*0501, DQA1*0401, and DQA1*0601 cannot be differentiated by DQ RFLP typing; DQA1*0501 is assumed because DQA1*0401 and DQA1*0601 are not associated with DQw2.
[c] Patients PSA and PT are either homozygous for DQw2 or possess a blank antigen in combination with DQw2; therefore, both alleles are considered DQw2-associated alleles. Both patients displayed an RFLP pattern consistent with the DQA1*0201 allele in addition to the DQA1*0501 allele pattern.
[d] RFLP pattern may represent either DQA1*0201 allele [38], or DQA1*0301; DQA1*0201 would be expected in this DQw2 patient.
[e] ND, not done.

DQB1 allele. Further characterization of the non-DQw2 alleles in these patients was not performed.

HLA-DQA1 Locus RFLP analysis of the DQA1 locus of eight HLA-DR3, -DQw2 patients with DH revealed an RFLP pattern consistent with the HLA-DR3 associated allele, DQA1*0501. Of interest, two of these patients also displayed a pattern consistent with a second DQw2-associated DQA1 allele, which is present predominately in HLA-DR7 subjects, DQA1*0201 [40-42]. In order to determine whether all patients with DH possess the DQB1*0201 allele in combination with the DQA1*0501 allele, regardless of their HLA-DR antigen expression, we also evaluated the DQA1 locus in two patients with DH who expressed HLA-DQw2 but not HLA-DR3. Although RFLP analysis of one of these patients did reveal a pattern consistent with the presence of DQA1*0501, the other non-HLA-DR3 patient had an RFLP pattern inconsistent with the presence of the DQA1*0501 allele (Table I). Thus although eight of eight HLA-DQw2, -DR3 patients displayed an RFLP pattern consistent with the DQA1*0501 allele, one of two HLA-DQw2, non-DR3 patients with DH did not possess this allele, suggesting that patients with DH can possess either form of the HLA-DQw2 heterodimer (DQB1*0201/DQA1*0501 or DQB1*0201/DQA1*0201), dependent upon their HLA-DR antigen association.

DISCUSSION

Recently, Scharf and co-workers and Sinha et al have substantiated the existence of unique subtypes of HLA antigens by reporting specific HLA class II DNA sequences of the DQB1 and DRB1 genes that are highly associated with the autoimmune skin disease pemphigus vulgaris [32,33]. Similar correlations between primary DNA sequence and disease susceptibility have been found in patients with IDDM [30,31]. These investigators have suggested that subtle changes in the HLA class II molecule may be important either in altering the antigen-binding capabilities of these immunoregulatory molecules or as specific markers of an HLA disease-susceptible haplotype [30-33].

In order to assess the possibility that subtle changes in HLA class II molecules may be operative in the pathogenesis of DH, we have characterized by DNA sequence and RFLP analysis the polymorphic second exons of the HLA-DQw2 genes found in patients with dermatitis herpetiformis. We have found that the DNA sequence of these regions is identical to that found in HLA-DQw2 normal subjects and that no distinct form of the HLA-DQw2 $\alpha\beta$ heterodimer is present in all patients with DH. The second exon of the HLA-DQ α and β chain genes is the region that is highly polymorphic and is thought to encode the antigen-binding region of the HLA molecule. Our data documenting no alteration in the second exon of both the HLA-DQ α and β chain genes suggests that molecularly unique subtypes of the HLA antigen, HLA-DQw2, do not appear to play a primary role in the pathogenesis of the autoimmune blistering skin disease dermatitis herpetiformis.

Clearly, the HLA-DQw2 antigens present in patients with DH are not associated with unique DNA sequences at the DQA1 or DQB1 loci; the fact remains, however, that virtually all patients with DH possess the DQw2 antigen compared with only 40% of normal controls. These data support the hypothesis that the HLA-DQw2 antigen is critical to, but not sufficient for, the development of dermatitis herpetiformis. The ubiquitous DQB2 allele at the DQ β chain locus supports the concept that unique residues on the DQ β chain may be critical elements within the HLA locus that act in concert with other gene products or environmental stimuli to cause DH. The DQB1*0201 allele is distinct from all other known DQ β chain alleles at eight sites, five of which are located in putative antigen binding sites, based on the model of Brown et al [47]. Of the unique residues located at putative antigen-binding sites, the positively charged lysine at position 71 carries the most significant charge difference from the other DQ β chain alleles, as noted by Kagnoff et al [39]. Conceivably, this uniquely charged residue could be responsible for alterations in antigen-binding capabilities, leading to immunoregulatory imbalance, which, in conjunction with additional factors, results in the development of DH. Of note, the DQB1*0201 allele contains an alanine residue at position 57, which, although not in a putative antigen-binding site, has been positively associated with the development of IDDM [30].

These findings are consistent with the DNA sequence analysis of HLA class II genes in patients with celiac disease, who have similar gastrointestinal lesions but no skin disease [39]. This suggests that the clinical differences between patients with celiac disease and those with DH are not due to genetic differences at either the α or β chain genes of the HLA-DQ locus.

Although patients with DH do not possess unique HLA-DQw2 molecules, as determined by analysis of the DQA1 and DQB1 loci, our findings do not exclude a role for HLA class II antigens in the pathogenesis of DH. The HLA-B8/-DR3/-DQw2 haplotype, and the ubiquitous DQB2 chain in particular, may function permissively, responsible for a state of immune hyperactivity to foreign or self antigens which, in conjunction with an environmental stimulus or the product of another gene(s), permits the development of dermatitis herpetiformis. The concept of a permissive state of immune hyperactivity conferred by certain HLA haplotypes is reinforced by the marked association of HLA-B8/-DR3/-DQw2 with other autoimmune diseases and the presence of in vitro immune defects in normal HLA-B8/-DR3 subjects [48-50]. It is also possible that the

HLA-DQw2 antigen in patients with DH may be altered in regions of the molecule other than the highly polymorphic first domain. This possibility appears unlikely, however, because these regions of the HLA molecule are highly conserved [23,24,26]. Alternatively, the HLA-DR region in patients with DH may encode a unique molecule that is responsible for the development of DH, although the lower association of DR3 with DH makes this less likely. Finally, the HLA-DQw2 antigen may simply be in linkage disequilibrium with the disease susceptibility locus. Continued investigation of the strong HLA associations in dermatitis herpetiformis will enhance our understanding of these general concepts of autoimmunity.

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REFERENCES

1. Hall RP: The pathogenesis of dermatitis herpetiformis: recent advances. J Am Acad Dermatol 16:1129–1144, 1987
2. Bodmer WF: The HLA system: structure and function. J Clin Pathol 40:948, 1987
3. Schlosstein L, Terasaki PI, Bluestone R, Pearson CM: High association of an HLA-A antigen, w27, with ankylosing spondylitis. N Engl J Med 288:704, 1973
4. Brewerton DA, Caffrey M, Hart FD, James DCO, Nicholls A, Sturrock RD: Ankylosing spondylitis and HL-A 27. Lancet I:904, 1973
5. Falchuk ZM, Rogentine GN, Strober W: Predominance of histocompatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. J Clin Invest 51:1602–1604, 1972
6. Stokes PL, Asquith P, Holmes GKT, Mackintosh P, Cooke WT: Histocompatibility antigens associated with adult coeliac disease. Lancet II:162–164, 1972
7. Tiwari JL, Terasaki PI: HLA and Disease Associations. Springer-Verlag, New York, 1985
8. Strominger JL: Biology of the human histocompatibility leukocyte antigen (HLA) system and a hypothesis regarding the generation of autoimmune diseases. J Clin Invest 77:1411, 1986
9. Batchelor JR, McMichael AJ: Progress in understanding HLA and disease associations. Br Med Bull 43:156, 1987
10. Keuning JJ, Pena AS, van Leewen A, van Hooff JP, van Rood JJ: HLA-DW3 associated with coeliac disease. Lancet I:506–508, 1976
11. Solheim BG, Ek J, Thune PO, Baklein K, et al: HLA antigens in dermatitis herpetiformis and coeliac disease. Tissue Antigens 7:57–59, 1976
12. Thomsen M, Platz R, Marks J, et al: Association of LD-8a and LD-12a with dermatitis herpetiformis. Tissue Antigens 7:60–62, 1976
13. Park MS, Terasaki PI, Ahmed AR, Zone J: The 90% incidence of HLA antigen (Te24) in dermatitis herpetiformis. Tissue Antigens 22:263–266, 1983
14. Katz SI, Falchuk ZM, Dahl MV, Rogentine GN, Strober W: HL-A8: a genetic link between dermatitis herpetiformis and gluten-sensitive enteropathy. J Clin Invest 51:2977–2980, 1972
15. Lawley TJ, Strober W, Yaoita H, Katz SI: Small intestinal biopsies and HLA types in dermatitis herpetiformis patients with granular and linear IgA skin deposits. J Invest Dermatol 74:9–12, 1980
16. Sachs JA, Leonard J, Awad J, et al: A comparative serological and molecular study of linear IgA disease and dermatitis herpetiformis. Br J Dermatol 118:759–764, 1988
17. Hashimoto K, Miki Y, Nishioka K, Nakata S, Matsuyama M: HLA antigens in dermatitis herpetiformis among Japanese. J. Dermatol 7:289–291, 1980
18. Sachs JA, Awad J, McCloskey D, et al: Different HLA associated gene combinations contribute to susceptibility for coeliac disease and dermatitis herpetiformis. Gut 27:515–520, 1986
19. Strober W: Immunogenetic factors. Dermatitis herpetiformis: the skin and the gut. Ann Intern Med 93:857–874, 1980
20. Hall RP, Sanders ME, Duquesnoy RJ, Katz SI, Shaw S: Alterations in HLA-DP and -DQ antigen frequency in patients with dermatitis herpetiformis. J Invest Dermatol 93:501–505, 1989
21. Hitman GA, Niven MJ, Festenstein H, et al: HLA class II alpha chain gene polymorphisms in patients with insulin dependent diabetes mellitus, dermatitis herpetiformis and celiac disease. J Clin Invest 79:609–615, 1987
22. Tosi R, Vismara D, Tanigaki N, et al: Evidence that celiac disease is primarily associated with a DC locus allelic specificity. Clin Immunol Immunopathol 28:395–404, 1983
23. Giles RC, Capra JD: Structure, function and genetics of human class II molecules. Adv Immunol 37:1–71, 1985
24. Auffray C, Strominger JL: Molecular genetics of the human major histocompatibility complex. Adv Hum Genet 15:197, 1986
25. The WHO nomenclature committee for factors of the HLA system: nomenclature for factors of the HLA system, 1989. Immunogenetics 31:131–40, 1990
26. Kostyu DD, Amos DB: The HLA complex: genetic polymorphism and disease susceptibility. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds.). The Metabolic Basis of Inherited Disease. McGraw Hill, New York, 225–249, 1989
27. Owen MJ, Crumpton MJ: The role of class I and class II antigens in T cell recognition. Br Med Bull 43:228–240, 1987
28. Goverman J, Hunkapiller T, Hood L: A speculative view of the multicomponent nature of T cell antigen recognition. Cell 45:475–484, 1986.
29. Greenberg LJ, Gray ED, Yunis EJ: Association of HL-A5 and immune responsiveness in vitro to streptococcal antigens. J Exp Med 141:935–943, 1975
30. Todd JA, Bell JI, McDevitt HO: HLA-DQβ gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329:599–604, 1987
31. Todd JA, Acha-Orbea H, Bell JI, et al: A molecular basis for MHC class II-associated autoimmunity. Science 240:1003–1009, 1988
32. Sinha AA, Brautbar C, Szafer F, et al: A newly characterized HLA DQβ allele associated with pemphigus vulgaris. Science 239:1026–1029, 1988
33. Scharf SJ, Friedmann A, Steinman L, Brautbar C, Erlich HA: Specific HLA-DQB and HLA-DRB1 alleles confer susceptibility to pemphigus vulgaris. Proc Natl Acad Sci, USA 86:6215–6219, 1989
34. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N: Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354, 1985
35. Mullis KB, Faloona FA: Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol 155:335–350, 1987
36. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA: Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–91, 1988
37. Horn GT, Bugawan TL, Long CM, Erlich HA: Allelic sequence variation of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes susceptibility. Proc Natl Acad Sci USA 85:6012–6016, 1988
38. Gyllensten UB, Erlich HA: Generation of single-stranded DNA by polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. Proc Natl Acad Sci USA 85:7652–7656, 1988
39. Kagnoff MF, Harwood JI, Bugawan TL, Erlich HA: Structural analysis of the HLA-DR, -DQ, and -DP alleles on the celiac disease-associated HLA-DR3 (DRw17) haplotype. Proc Natl Acad Sci USA 86:6274–6278, 1989
40. Maeda M, Inoko H, Ando A, Uryu N, Tsuji K: HLA-D typing of heterozygotes using restriction fragment length polymorphism in the HLA-DQ gene region on the basis of standard band patterns derived from HLA homozygotes. Hum Immunol 25:195–205, 1989
41. Uryu N, Maeda M, Ota M, Tsuji K, Inoko H: A simple and rapid method for HLA-DRB and -DQB typing by digestion of PCR-amplified DNA with allele specific restriction endonucleases. Tissue Antigens 35:20–31, 1990

42. Maeda M, Murayama N, Ishii H, Ota M, Tsuji K, Inoko H: A simple and rapid method for HLA-DQA1 genotyping by digestion of PCR-amplified DNA with allele specific restriction endonucleases. Tissue Antigens 34:290–298, 1989

43. Mittal KK, Mickey MR, Singal ER, Terasaki P: Serotyping for homotransplantation. XVIII. Regimen of microdrop lymphocyte cytotoxicity test. Transplantation 6:913–927, 1968

44. Hall RP, Ward FE, Wenstrup RJ: An HLA class II restriction fragment length polymorphism (RFLP) in patients with dermatitis herpetiformis: association with HLA-DP phenotype. J Invest Dermatol 95:172–177, 1990

45. Scharf SJ, Horn GT, Erlich HA: Direct cloning and sequence analysis of enzymatically amplified genomic sequences. Science 233:1076–1078, 1986

46. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5466, 1977

47. Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC: A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. Nature 332:845–850, 1988

48. Lawley TJ, Hall RP, Fauci AS, Katz SI, Hamburger MI, Frank MM: Defective Fc-Receptor functions associated with the HLA-B8/DRw3 haplotype. Studies in patients with dermatitis herpetiformis and normal subjects. N Engl J Med 304:185–192, 1981

49. Cunningham-Rundles S, Cunningham-Rundles C, Pollack NS, Good RA, Dupont B: Response to wheat antigen in in vitro lymphocyte transformation among HLA-B8 positive normal donors. Transplant Proc 10:977–979, 1978

50. McCombs CC, Michalski JP: Lymphocyte abnormality associated with HLA-B8 in healthy young adults. J Exp Med 156:936–941, 1982